Remarks

Claim 1 is pending.

The applicant wishes to thank the examiner for indicating that all of the rejections under 35 USC §112 are withdrawn. Two rejections remain – both are under 35 USC §103(a)

Regarding section 7 of the office action:

Claim 1 stands rejected as being unpatentable over Maliga (U.S. Patent No. 5,877,402) and Davies (WO 90/11770). The applicant respectfully traverses this rejection.

Maliga '402 relates generally to plastid transformation and uses a selectable marker for herbicide resistance. (The Abstract, for example, of Maliga '402 refers to a "non-lethal" selectable marker gene.) Maliga '402 is not cited as teaching or suggesting that anti-microbial peptides (AMPs) can be used for plastid transformation.

Davies relates to AMPs, which are cytotoxic. Davies is limited to nuclear transformation. Davies is not cited as teaching or suggesting that these AMPs can be used to successfully transform plastids.

Nothing in Maliga and Davies, taken alone or in combination, teaches or suggests that AMP-expressing vectors can be used with the expectation of successfully transforming plastids. Following are paragraphs from the subject application, which published as US 20040093636 [underlining added]:

> [0015] Plastid Transformation: To date, plastid transformation, particularly has enabled generation of herbicide (Daniell et al., 1998), insect resistant crops (Kota et al., 1999; McBride et al., 1995; DeCosa et al., 2000) and production of pharmaceutical proteins (Guda et al., 2000; Staub et al., 2000). Plastid transformation was selected because of several advantages over nuclear transformation (Daniell, 1999 A, B; Bogorad, 2000; Heifetz, 2000). With concern growing about outcrossing of genetically altered genes, it should be noted that plastid expressed genes are maternally inherited in most crops. Gene containment is possible when foreign genes are engineered via the plastid genome, which prevents pollen transmission in crops that maternally inherit the plastid genome. Because a majority of crop plants inherit their plastid genes maternally, the foreign genes do not escape into the environment. Although pollen from plants that exhibit maternal inheritance contain metabolically active plastids, the plastid DNA is lost during pollen maturation (Helfetz, 2000). Despite the potential advantage of plastid reproduction of AMPs, it was not obvious that AMPs would be produced in this manner. Prior to the patent application there were no published reports of expression of AMPs in plant

plastids. Non-obviousness of the disease resistance. Several foreign genes have been expressed within plastids to introduce novel traits including herbicide resistance or insect resistance. However, all of these foreign proteins, without exception, function within plastids. For example, herbicides target proteins or enzymes present within plastids. When engineered plastids are consumed by target insects; insecticidal proteins are released inside the insect gut.

[0016] However, in order to use the chloroplast compartment to engineer disease resistance, it was necessary to export foreign proteins into the cytosol where phytopathogens colonize. Therefore, it was not obvious to engineer the plastid genome to confer disease resistance. There are no prior reports or suggestions in the literature that plastid genome could be engineered to confer disease resistance. Also, it is known in the art that antimicrobial peptides are toxic to plant chloroplasts because of the charge on the chloroplast membranes. However, this invention teaches that transgenic plastids expressing antimicrobial peptides rupture at the site of infection upon cell death. Release of large amounts of the antimicrobial peptide prevent the spread of the phytopathogen. Thus, the present invention confirms a novel and unobvious solution to combat phytopathogens that is previously unknown and contrary to all current understanding of chloroplast biology.

[0017] Most importantly, small peptides are not stable inside living cells and are highly susceptible to proteolytic degradation. For this reason, small peptides are usually produced as fusion proteins with larger peptides in biological systems. Megainin type peptides are chemically synthesized and never made in biological systems for that reason. Therefore, it was not obvious to express a small peptide of a few amino acids within plastids. Successful expression of this antimicrobial peptide was not anticipated but this invention opens the door for expression of several small peptides within plastids, including hormones.

The foregoing should have the effect of an expert declaration by Dr. Daniell, an expert in this field, who attested to (by signing the required oath/declaration for) this application. Thus, there is evidence on the record that puts Maliga and Davies in context and shows that these references are consistent with how the subject application discusses the state of the art: there are no prior reports or suggestions in the literature that plastid genome could be engineered to confer disease resistance.

The office action states on page 4 that the applicant has not produced evidence of unexpected results. However, Example 1 of the subject application shows that plants (plastids) were successfully transformed and produced MSI-99 (a type of magainin) "at levels high enough to provide upwards of 96% inhibition of growth against *Pseudomons syringae*, a major plant

pathogen." Considering the state of the art as explained above, these results are *very* surprising and long sought after. As explained above, AMPs were known in the art to be toxic to plant chloroplasts because of the charge on the chloroplast membranes. Thus, it should be clear that the vectors as claimed heretofore had no expected utility.

Furthermore, if one considers the apparent origin of chloroplasts, which are commonly thought to have been once free-living cyanobacteria that became endosymbionts (*see e.g.* "Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reaveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus," Martin *et al.* PNAS (2002) vol. 99, no. 19, 12246-12251), it should be even more clear that the above results were highly surprising. AMPs are lytic peptides that disrupt bacterial membranes! There was no expectation that plastid transmformation (via a suitable vector as claimed) could result in AMP generation "at levels high enough to provide upwards of 96% inhibition of growth against *Pseudomons syringae*, a major plant pathogen."

Thus, one would not expect a magainin (or any other AMP) to "work" in a plastid. The obviousness rejection relies on one reference teaching a magainin and one reference teaching plastid transformation with a herbicide resistance gene. There is simply no support to bridge the large gap between these two references, and until the subject invention, one skilled in the art would not look across this gap and expect to successfully cross it. This rejection does not take into account the perceived problems in the art, such as AMPs/lytic peptides being known to be toxic to plant chloroplasts.

The office action also implies that the selection of a magainin to put in the vectors was obvious. There was no expectation that such constructs would be useful for successfully transforming plastids, and AMP-transformed plastids had never previously been achieved. Not only is this rejection inconsistent with the state of the art as explained above, it is contrary to and inconsistent with statements made on page 6 of the office action dated March 25, 2003, on parent application serial number 09/807,720:

> Applicant urges that DeGray et al, enclosed, demonstrates the expression of MSI-99 in transformed plastids. Applicant urges that Okamoto et al and Allefs et al, cited in the prior Office action, teach insertion of an AMP into the nuclear genome of plants, which negatively affected their ability to be transcribed and retained in the cytosol (respons pg

11). This is not found persuasive [as] Okamoto et al and Allefs et al teach that the expression of other antimicrobial proteins is unpredictable. The specification and DeGray et al only show that MSI-99 can be expressed and do not overcome the unpredictability of other AMPs.

The position that a successful magainin plastid transformation vector would have been obvious is undermined by Okamoto and Allefs, which were previously cited as teaching that expression of AMPs is unpredictable. One skilled in the art had no expectation that a magainin could be functionally produced by plastids as discussed above. Thus, specifying a magainin as claimed adds another layer of nonobviousness to the presently claimed invention. There was no basis in the art for selecting these AMPs out of all the possibilities. In addition, to say that the subject vectors were produced using the teachings of the art involves hindsight reconstruction, as there was no prior motivation to make them, and there was no expectation of their success.

Regarding section 8 of the office action:

The addition of Smith (WO 99/06564) does nothing to overcome the limitations of Maliga, as discussed above (or Davies). Thus, the applicant respectfully traverses this obviousness rejection of claim 1.

Smith relates generally to magainin and PGL-type AMPs and has a specification of nearly 50 pages. Of those nearly 50 pages, two short paragraphs are cited as rendering the subject invention obvious. These paragraphs do nothing more than state a goal of AMP production in a plastid. However, as explained above, there were many perceived obstacles to successfully doing so. Smith provides nothing to bridge the gap. On page 9, Smith cites a reference relating to herbicide resistance and plastid transformation. As discussed above, this is what Maliga provides, and the applicant does not dispute that this type of plastid transformation was successful in the art. However, Smith, taken alone or in combination with Maliga, does not offer an expectation of success, which is required for a proper obviousness rejection.

As discussed above, Example 1 of the subject application provides unexpected results. These results were not expected in light of Smith and Maliga, especially when considered in light of the state of the art as a whole, as attested to in the subject specification. The additional comments provided in response to section 7 of the office action are equally applicable to this rejection and should be considered as such.

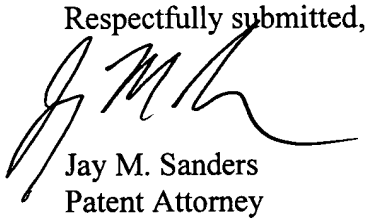
In light of all the foregoing, the withdrawal of both obviousness rejections is respectfully requested.

The applicant believes that this application is in condition for allowance, and such action is earnestly solicited.

The Assistant Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 and 1.17 as required by this paper to Deposit Account 19-0065.

The applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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Attachment: Petition and Fee for Extension of Time Under 37 CFR §1.136(a)